## Chemical Speciation of Radionuclide Uptake by Bacteria Using X-ray Microscopy

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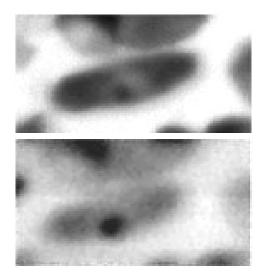
**Introduction**: Microorganisms in the natural environment may interact with radionuclide and toxic metal contaminants through (i) sorption, (ii) intracellular accumulation, and (iii) alteration of chemical speciation. These interactions may retard or enhance the mobility of the contaminants by precipitation reactions, biocolloid formation, or production of more soluble species. In addition, current and planned radioactive waste repository environments, such as deep subsurface halite and granite formations, harbor microorganisms. Life processes in these environments are considered extreme relative to the near-surface terrestrial environment; there is a paucity of information on the biotransformation of radionuclides by microorganisms present in such environments <sup>1,2</sup>. We are using spectromicroscopy to elucidate the functional groups associated with the bacterial cell that are involved in uptake of uranium, first by common soil microbes followed by microorganisms relevant to radioactive waste repositories.

**Methods and Materials**: Initial data has been gathered from C K-edge XANES of cells before and after exposure to the radionuclide. The soil bacteria analyzed include *Pseudomonas fluorescens* and *Bacillus subtilis* at pH 5; at this pH the carboxyl groups on the cell wall should be ionized and available to bind cations. Bacterial cells were dried on silicon nitride grids. Uranium standards were analyzed to assist in the interpretation of the functional groups involved in the complexation with the cell; these standards included uranyl nitrate, uranyl carbonate, and uranyl acetate.

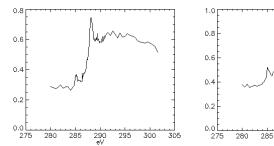
**Results**: *P. fluorescens* washed in DI water and exposed to uranyl nitrate at pH 5 resulted in significant accumulation of uranium (92  $\mu$ g U mg<sup>-1</sup> dry cells). Scanning transmission x-ray microscopy (STXM) of the cells showed strong absorbance at the C K-edge (288 eV) due to the 1s- $\pi$ \* resonance associated with the C=O bonds (**Figure 1** top). Below the C-edge there is weaker absorption, with a distinct area of strong sorption within the boundary of the cell's margin (**Figure 1** bottom). This region is rich in inorganic material and may be enriched with uranium. **Figure 2** shows the C K-edge XANES for *P. fluorescens* before and after exposure to uranium. There is no difference in the C=O absorption peak at 288 eV, however, with the addition of U a broad peak at 293 eV is consistently observed; this may be due to influence of U on the C-O  $\sigma$ \* resonance and changes in the C-H bonding environment.

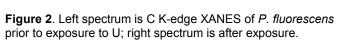
**Conclusions**: Carbon K-edge XANES analysis of bacterial cells before and after exposure to U reveals features in the spectra above 290 eV that may be due to U uptake. STXM of the bacterial cells exposed to uranium will be extended to analysis at the oxygen K-edge; here we have found unique XANES features between 535 - 545 eV for uranyl nitrate standards.

**References**: <sup>1</sup>A.J. Francis, J.B. Gillow, C.J. Dodge, M. Dunn, K. Mantione, B.A. Strietelmeier, M.E. Pansoy-Hjelvik, and H.W. Papenguth, Radiochim. Acta **82**, 347 (1998). <sup>2</sup>J.B. Gillow, M. Dunn, A.J. Francis, D.A. Lucero, and H.W. Papenguth, Radiochim. Acta 2000 – in press.



**Figure 1.** Top micrograph shows *P. fluorescens* bacterium exposed to uranyl nitrate imaged at the C edge (288 eV); bottom is imaged below the C edge.





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